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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
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10/574,551

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Philip Buzby

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EXAMINER

BERTAGNA, ANGELA MARIE

ART UNIT

PAPER NUMBER

1637

MAIL DATE

DELIVERY MODE

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PAPER

**Please find below and/or attached an Office communication concerning this application or proceeding.**

The time period for reply, if any, is set in the attached communication.

### Office Action Summary

**Application No.**

10/574,551

**Applicant(s)**

BUZBY, PHILIP

**Examiner**

ANGELA BERTAGNA

**Art Unit**

1637

**Period for Reply** -- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

**Status**

- 1) ☒ Responsive to communication(s) filed on 30 March 2006.
- 2a) ☐ This action is **FINAL**. 2b) ☒ This action is non-final.
- 3) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

**Disposition of Claims**

- 4) ☒ Claim(s) 1-7,9,10,13-16,18,21,23,24,26-30 and 32 is/are pending in the application.
- 4a) Of the above claim(s) \_\_\_\_\_ is/are withdrawn from consideration.
- 5) ☐ Claim(s) \_\_\_\_\_ is/are allowed.
- 6) ☒ Claim(s) 1-7,9,10,13-16,18,21,23,24,26-30 and 32 is/are rejected.
- 7) ☒ Claim(s) 24 is/are objected to.
- 8) ☐ Claim(s) \_\_\_\_\_ are subject to restriction and/or election requirement.

**Application Papers**

- 9) ☒ The specification is objected to by the Examiner.
- 10) ☒ The drawing(s) filed on 30 March 2006 is/are: a) ☒ accepted or b) ☐ objected to by the Examiner.
- Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).
- Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).
- 11) ☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

**Priority under 35 U.S.C. § 119**

- 12) ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
- a) ☐ All b) ☐ Some \* c) ☐ None of:
1. ☐ Certified copies of the priority documents have been received.
  2. ☐ Certified copies of the priority documents have been received in Application No. \_\_\_\_\_.
  3. ☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

\* See the attached detailed Office action for a list of the certified copies not received.

**Attachment(s)**

- 1) ☒ Notice of References Cited (PTO-892)
- 2) ☐ Notice of Draftsperson's Final Drawing Review (PTO-948)
- 3) ☒ Information Disclosure Statement(s) (PTO/SB/08)  
Paper No(s)/Mail Date 11/19/07.
- 4) ☐ Interview Summary (PTO-413)  
Paper No(s)/Mail Date \_\_\_\_\_.
- 5) ☐ Notice of Informal Patent Application
- 6) ☐ Other: \_\_\_\_\_.

### **DETAILED ACTION**

#### ***Remarks***

1. Applicant's submission of a preliminary amendment on March 30, 2006 is acknowledged. Claims 1-7, 9, 10, 13-16, 18, 21, 23, 24, 26-30, and 32 are currently pending and will be examined on the merits.

#### ***Information Disclosure Statement***

2. Applicant's submission of an Information Disclosure Statement on November 19, 2007 is acknowledged. A signed copy is enclosed.

#### ***Drawings***

3. The drawings filed on March 30, 2006 are acceptable.

#### ***Specification***

4. The disclosure is objected to because of the following informalities: Nucleic acid sequences greater than ten nucleotides in length are recited at pages 29-30 of the specification and also in Figure 1. These sequences must be identified by the appropriate SEQ ID NO: (see 37 CFR 1.821-1.825). Regarding the nucleic acid sequences present in Figure 1, the sequence identifier may appear in the drawing or in the "Brief Description of the Drawings" section (see MPEP 2422.02).

Appropriate correction is required.

The disclosure is objected to because it contains an embedded hyperlink and/or other form of browser-executable code. Applicant is required to delete the embedded hyperlink and/or other form of browser-executable code. See MPEP § 608.01.

### ***Claim Objections***

5. Claim 24 is objected to under 37 CFR 1.75(c), as being of improper dependent form for failing to further limit the subject matter of a previous claim. Applicant is required to cancel the claim(s), or amend the claim(s) to place the claim(s) in proper dependent form, or rewrite the claim(s) in independent form. Claim 24 recites that the acyclo nucleoside terminator of claim 23 comprises a detectable label. Claim 23 depends from claim 1, which recites that the terminator contains a detectable label in line 7. Accordingly, claim 24 fails to further limit the method of claim 23, because claim 1 recites that the terminator comprises a detectable label.

### ***Claim Rejections - 35 USC § 112, 2<sup>nd</sup> paragraph***

6. The following is a quotation of the second paragraph of 35 U.S.C. 112:

The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 1-7, 9, 10, 13-16, 18, 21, 23, 24, 26-30, and 32 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claims 1 and 26 are indefinite, because they recite the limitation "the quantity of pyrophosphate" in line 6. There is insufficient antecedent basis for this limitation in the claim. There is sufficient antecedent basis for "the quantity of inorganic pyrophosphate".

Claims 2-7, 9, 10, 13-16, 18, 21, 23, 24, and 27-30 are indefinite, because they depend from claim 1 or claim 26.

Claims 4 and 29 are further indefinite, because the first step recites adding an enzyme to the purified reaction product, while the second step recites incubating the enzyme with the nucleic acid synthesis product to degrade the residual component. As a result, it is not clear whether the enzyme is added to the purified reaction product, the nucleic acid synthesis product, or both products.

Claim 30 is further indefinite, because it recites the limitation "the inorganic pyrophosphatase" in line 2. There is insufficient antecedent basis for this limitation in the claim. There is sufficient antecedent basis for "the pyrophosphate removing enzyme".

Claim 32 is indefinite, because it recites the limitation "the pyrophosphatase" in line 7. There is insufficient antecedent basis for this limitation in the claim. There is sufficient antecedent basis for "the inorganic pyrophosphatase".

#### ***Claim Rejections - 35 USC § 103***

7. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negated by the manner in which the invention was made.

8. Claims 1-7, 9, 10, 13-16, 21, and 26-30 are rejected under 35 U.S.C. 103(a) as being unpatentable over Kwok et al. (US 6,180,408 B1) in view of Tabor et al. (US 5,498,523).

These claims are drawn to methods of inhibiting misincorporation of a terminator nucleotide in a single base extension reaction. The methods comprise treating a nucleic acid synthesis product with an inorganic pyrophosphatase to degrade inorganic pyrophosphate and thereby reduce the occurrence of pyrophosphorolysis.

Kwok teaches a homogenous genotyping method comprising PCR amplification, single base extension, and fluorescence polarization detection (see abstract, column 6, line 55 – column 7, line 10, and Figure 2 for a general description).

Regarding claims 1 and 26, the method of Kwok comprises:

(a) providing a product of a nucleic acid synthesis reaction comprising a nucleic acid template and a quantity of inorganic pyrophosphate (column 10, lines 29-47, where the PCR amplification step inherently generates inorganic pyrophosphate)

(b) purifying the nucleic acid synthesis product to obtain a purified reaction product (column 10, lines 48-58)

(c) combining the purified reaction product with a primer, a labeled terminator nucleoside, and a polymerase (column 10, line 60 - column 11, line 2)

(d) extending the primer by addition of the labeled terminator in a single base extension reaction (column 11, lines 2-5).

Regarding claims 2 and 27, the PCR amplification product obtained in the method of Kwok contains residual primers and nucleotides (see column 10, lines 48-58).

Regarding claims 3 and 28, Kwok teaches incubating the nucleic acid synthesis product with an exonuclease and an alkaline phosphatase to degrade the residual primers and nucleotides and then inactivating the enzymes (column 10, lines 48-58).

Regarding claims 5, 6, and 30, Kwok teaches enzyme inactivation (col. 10, lines 55-56).

Regarding claim 7, Kwok teaches that the detectable label is a fluorescent label (see column 10, line 67 and column 11, lines 9-55).

Regarding claims 9 and 10, Kwok teaches detecting the label using fluorescence polarization (column 11, lines 9-55).

Regarding claim 13, Kwok teaches that the alkaline phosphatase is a bacterial alkaline phosphatase (column 6, lines 63-64, where HK thermolabile phosphatase is a bacterial alkaline phosphatase).

Regarding claim 14, Kwok teaches that the alkaline phosphatase is shrimp alkaline phosphatase (column 10, line 51).

Regarding claims 15 and 16, Kwok teaches that the exonuclease is exonuclease I or mung bean exonuclease (see column 6, lines 60-62 and column 10, line 52).

Regarding claim 21, Kwok teaches that the steps are performed in a single reaction container (column 3, line 65 - column 4, line 5).

Kwok does not teach incubating the nucleic acid synthesis product or the purified reaction product with an inorganic pyrophosphatase or a pyrophosphate removing enzyme to reduce the quantity of inorganic pyrophosphate present in the nucleic acid synthesis product or the purified reaction product.

Tabor teaches a method for conducting PCR in the presence of an inorganic pyrophosphatase (see column 3, lines 3-13 and column 4, lines 30-67). Regarding claims 1 and 26, Tabor teaches that the inclusion of an inorganic pyrophosphatase in the amplification reaction inhibits pyrophosphorolysis, which is detrimental to the primer extension step (column

3, lines 14-25). Tabor teaches that inhibiting pyrophosphorolysis improves the efficiency of the reaction (column 3, lines 24-27 and column 4, lines 63-67). Tabor teaches that these benefits are also applicable to primer extension reactions and sequencing reactions (column 3, lines 14-33 and lines 55-59).

It would have been *prima facie* obvious for one of ordinary skill in the art at the time of invention to apply the teachings of Tabor to the method taught by Kwok. An ordinary artisan would have been motivated to include an inorganic pyrophosphatase in the PCR amplification and primer extension steps of the method taught by Kwok, since Tabor taught that inorganic pyrophosphatase degrades inorganic pyrophosphate produced by these reactions, thereby improving the efficiency of the reaction by inhibiting the detrimental pyrophosphorolysis reaction (column 3, lines 14-25). An ordinary artisan would have had a reasonable expectation of success in doing so, since Tabor taught that inorganic pyrophosphatase was commercially available (column 4, lines 51-56). Finally, regarding claims 4 and 29, it would have been *prima facie* obvious to add the exonuclease and alkaline phosphatase together with or after the addition of the inorganic pyrophosphatase, since section 2144.04 IV C of the MPEP states that any order of mixing ingredients is *prima facie* obvious. Thus, absent any secondary considerations, the methods of claims 1-7, 9, 10, 13-16, 21, and 26-30 are *prima facie* obvious over Kwok in view of Tabor.



9. Claims 18, 23, and 24 are rejected under 35 U.S.C. 103(a) as being unpatentable over Kwok et al. (US 6,180,408 B1) in view of Tabor et al. (US 5,498,523) and further in view of Jack et al. (WO 01/23411 A2).

These claims are drawn to the method of claim 1, wherein the terminator is an acyclo nucleoside terminator and the method is conducted using a polymerase that has a higher affinity for an acyclo nucleoside terminator than for a dideoxy terminator.

The combined teachings of Kwok and Tabor result in the method of claims 1-7, 9, 10, 13-16, 21, and 26-30, as discussed above.

These references do not teach that the terminator is an acyclo nucleoside terminator and the method is conducted using a polymerase that has a higher affinity for an acyclo nucleoside terminator than for a dideoxy terminator.

Jack teaches methods and compositions for improving the incorporation of chain terminating nucleotides by DNA polymerases (see abstract and page 9, line 30 - page 10, line 10). Regarding claims 18, 23, and 24, Jack teaches that dye-labeled acyclo-NTPs are more readily incorporated by Family B archaeon DNA polymerases, such as Vent, Pfu, Deep Vent, and 9N, than dye-labeled ddNTPs (page 18, line 9 – page 19, line 14). Jack also teaches that increasing the efficiency of nucleotide terminator incorporation reduces costs, decreases background, and increases assay sensitivity (page 25, line 31 - page 26, line 2).

It would have been *prima facie* obvious for one of ordinary skill in the art at the time of invention to apply the teachings of Jack to the method taught by Kwok. An ordinary artisan would have been motivated to substitute dye-labeled acyclo NTPs and a Family B archaeon DNA polymerase for the dye-labeled ddNTPs and AmpliTaq-FS taught by Kwok, since Jack

taught that dye-labeled acyclo NTPs were more efficiently incorporated by a Family B archaeon DNA polymerase than ddNTPs (page 18, line 9 - page 19, line 14). Since Jack taught the more efficient terminator incorporation reduced costs, decreased background, and improved sensitivity (page 25, line 31 - page 26, line 2), an ordinary artisan would have been particularly motivated to substitute dye-labeled acyclo NTPs and a Family B archaeon DNA polymerase in the method of Kwok in order to obtain these advantages. An ordinary artisan would have had a reasonable expectation of success in using dye-labeled acyclo NTPs and a Family B DNA polymerase in the method of Kwok, since Jack taught that they were suitable for single base extension methods, such as the single base extension method taught by Kwok (page 26, line 25 – page 27, line 2). Thus, the methods of claims 18, 23, and 24 are *prima facie* obvious over Kwok in view of Tabor and further in view of Jack.

10. Claim 32 is rejected under 35 U.S.C. 103(a) as being unpatentable over Kwok et al. (US 6,180,408 B1) in view of Tabor et al. (US 5,498,523) and further in view of Jack et al. (WO 01/23411 A2).

Claim 32 is drawn to a method for inhibiting misincorporation of a terminator in a single base extension assay. The method comprises combining a nucleic acid template, a primer, an inorganic pyrophosphatase, a polymerase, and an acyclo nucleoside terminator and extending the primer via addition of the acyclo nucleoside terminator, wherein the inorganic pyrophosphatase inhibits pyrophosphorolysis.

Kwok teaches a homogenous genotyping method comprising PCR amplification, single base extension, and fluorescence polarization (see abstract, column 6, line 55 – column 7, line 10, and Figure 2 for a general description).

Regarding claim 32, the method of Kwok comprises:

(a) combining a nucleic acid template, a primer, a nucleoside terminator, and a polymerase to form a mixture substantially free of deoxynucleotide triphosphates (column 10, line 60 - column 11, line 2)

(b) incubating the mixture under conditions sufficient to extend the primer by addition of the terminator (column 11, lines 2-5).

Kwok does not teach that the mixture further includes an inorganic pyrophosphatase in order to inhibit pyrophosphorolysis. Kwok also does not teach the use of an acyclo nucleoside terminator.

Tabor teaches a method for conducting PCR in the presence of an inorganic pyrophosphatase (see column 3, lines 3-13 and column 4, lines 30-67). Tabor teaches that the inclusion of an inorganic pyrophosphatase in the amplification reaction inhibits pyrophosphorolysis, which is detrimental to the primer extension step (column 3, lines 14-25). Tabor teaches that inhibiting pyrophosphorolysis improves the efficiency of the reaction (column 3, lines 24-27 and column 4, lines 63-67). Tabor teaches that these benefits are also applicable to primer extension reactions and sequencing reactions (column 3, lines 14-33 and lines 55-59).

Tabor does not teach the use of acyclo nucleoside terminators.

Jack teaches methods and compositions for improving the incorporation of chain terminating nucleotides by DNA polymerases (see abstract and page 9, line 30 - page 10, line

10). Jack teaches that dye-labeled acyclo-NTPs are more readily incorporated by Family B archaeon DNA polymerases, such as Vent, Pfu, Deep Vent, and 9N, than dye-labeled ddNTPs (page 18, line 9 – page 19, line 14). Jack also teaches that increasing the efficiency of nucleotide terminator incorporation reduces costs, decreases background, and increases assay sensitivity (page 25, line 31 - page 26, line 2).

It would have been *prima facie* obvious for one of ordinary skill in the art at the time of invention to apply the teachings of Tabor and Jack to the method taught by Kwok. An ordinary artisan would have been motivated to include an inorganic pyrophosphatase in the PCR amplification and primer extension steps of the method taught by Kwok, since Tabor taught that inorganic pyrophosphatase degrades inorganic pyrophosphate produced by these reactions, thereby improving the efficiency of the reaction by inhibiting the detrimental pyrophosphorolysis reaction (column 3, lines 14-25). An ordinary artisan would have had a reasonable expectation of success in doing so, since Tabor taught that inorganic pyrophosphatase was commercially available (column 4, lines 51-56). An ordinary artisan also would have been motivated to substitute dye-labeled acyclo NTPs and a Family B archaeon DNA polymerase for the dye-labeled ddNTPs and AmpliTaq-FS taught by Kwok, since Jack taught that dye-labeled acyclo NTPs were more efficiently incorporated by a Family B archaeon DNA polymerase than ddNTPs (page 18, line 9 - page 19, line 14). Since Jack taught the more efficient terminator incorporation reduced costs, decreased background, and improved sensitivity (page 25, line 31 - page 26, line 2), an ordinary artisan would have been particularly motivated to substitute dye-labeled acyclo NTPs and a Family B archaeon DNA polymerase in the method of Kwok in order to obtain these advantages. An ordinary artisan would have had a reasonable expectation of

success in using dye-labeled acyclo NTPs and a Family B DNA polymerase in the method of Kwok, since Jack taught that they were suitable for single base extension methods, such as the single base extension method taught by Kwok (page 26, line 25 – page 27, line 2). Thus, the method of claim 32 is *prima facie* obvious over Kwok in view of Tabor and further in view of Jack.

### ***Conclusion***

11. No claims are currently allowable.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to ANGELA BERTAGNA whose telephone number is (571)272-8291. The examiner can normally be reached on M-F, 7:30 - 5.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Benzion can be reached on 571-272-0782. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see <http://pair-direct.uspto.gov>. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

Amb

/Cynthia Wilder/  
Patent Examiner, Art Unit 1637